



## WATER RESOURCES RESEARCH GRANT PROPOSAL

**Project ID:** 2003KY21B

**Title:** Effects of a waterborne herbicide, Atrazine, on the auditory physiology of fish

**Project Type:** Research

**Focus Categories:** Agriculture, Toxic Substances, Water Quality

**Keywords:** herbicides, neurotoxicity, auditory physiology

**Start Date:** 03/01/2003

**End Date:** 02/28/2004

**Federal Funds Requested:** \$15000.00

**Matching Funds:** \$30000.00

**Congressional District:** Kentucky 6th

**Principal Investigators:** Yan, Hong Y.

**Abstract:** Atrazine (2-chloro-4-ethylamino-6-isopropylamine-1,3,5-triazine) is a widely used herbicide in the world and in the United States. The half-life of atrazine in loamy soils ranges from 60 to 150 days and half-life itself can extend to 660 days in anaerobically incubated sand clay sediment. This chemical property makes it susceptible to leaching and runoff, especially during heavy rain. Once it enters into the water, the degradation slows down and the half-life in reservoirs may last up to 2 years. In Kentucky alone, in 1999 a total of 432,000 kg of atrazine was applied to corn and sorghum fields across the state. A total of 1,562,000 acres of farmland in Kentucky was treated with atrazine and more than 1,543,000 acres where run-off concentration of atrazine was found to exceed the 3 ppb maximum contaminant level as set by the US Environmental Protection Agency. Because of easy runoff, atrazine is the most commonly detected herbicide, with 540 detections in 2,330 samples, in Kentucky. The atrazine contamination to ground water and springs is reported to have occurred in all Kentucky Basin Management Units across the state. Clearly, water borne atrazine contamination is a statewide problem to both humans and aquatic animals and deserves great attention. Long-term (chronic) oral exposure to atrazine on rats have shown a variety of adverse effects to heart, lung, liver, kidney, spleen, adrenal glands and brain. Recently, it is demonstrated that atrazine disrupts endocrinological function of amphibians and induces hermaphroditism (simultaneous existence of both male and female reproductive organs) in males and

hence disrupts normal reproduction. The abnormal rate ranges from 10 to 92% from frog samples collected across the U.S. It is known that atrazine is widely distributed in Kentucky waters, it is imperative that studies be conducted to investigate how this herbicide is impacting the overall well being of fishes of Kentucky. The thrust of the proposed research is to measure changes of auditory physiology of fathead minnow after exposed to atrazine of certain concentration and duration. Atrazine has been implicated in causing hypothyroidism, i.e., suppressed thyroid gland function, in mammalian animals and subsequently affects hearing ability of animals. Preliminary results from my laboratory demonstrates that under induced hypothyroidism condition, hearing thresholds of fathead minnows are elevated and enlargement of follicles of thyroid gland, i.e., thyroid goiter, is detected. The auditory system is one of the primary sensory modalities used by animals to sense the acoustic landscape in order to avoid predators, to detect prey, and to respond to mating signals. A hearing impaired fish does not have chance to survive even if the pollutants in its environment are under sublethal levels. Therefore, changes of auditory physiology can be used as an effective method to assay the impacts from exposure to atrazine. Three sets of experiments are designed to test the hypothesis that atrazine can modulate hearing ability of fathead minnow, a widely distributed fish species in the state of Kentucky, and hence an effective bio-indicator species for the study. The first set of experiments will subject fathead minnow to a combinations of 5 atrazine concentrations (0.01, 0.5, 1.0, 2.0. and 3.0 ppb) and from 1-10 weeks of exposure duration. Ten experimental fish and 5 control fish will be sampled at the end of each week. Plasma T4 (thyroxine) and T3 (triiodothyronine) will be assayed by radioimmunoassay (RIA) method and follicle size of thyroid gland will be measured by histological sections. The results of the first phase experiment will provide the needed information on the atrazine concentration and exposure duration that induces hypothyroidism. The second set of experiments will use the best combination of atrazine concentration and exposure duration data obtained from the first experiments to investigate how atrazine-induced hypothyroidism impacts changes of hearing ability of fathead minnow. This is achieved by measuring hearing threshold shifts with the use of an electrophysiological recording protocol, the auditory brainstem response (ABR), an electrophysiological technique on recording acoustically evoked brainwaves from fish, which was first pioneered in my laboratory. The third set of experiments is to test the hypothesis if atrazine can be transmitted vertically from female fish, i.e., mother, to next generation through the eggs. Female fish exposed to the right combination of atrazine concentration and duration (Exposed mothers) will be spawned with a normal (un-exposed) male, Normal females (Clean mothers) will also be spawned with a normal (un-exposed) male. Fertilized embryos from each type of mother are divided into two subgroups and are either subjected to atrazine exposure (Exposed offspring) or clean water (Clean offspring). Such an arrangement results in four combinations of offspring (Exposed mothers, exposed offspring; Exposed mothers, clean offspring; Clean mothers, exposed offspring; Clean mothers, clean offspring). Hearing ability of 4-month-old offspring will be assessed. By using the data from Clean mothers, clean offspring as baseline data, the data from the other three treatment groups will provide needed information to support or refute the hypothesis that atrazine can be vertically transmitted from mothers to offspring.

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